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Introduction of Citrus Germplasm into Florida[1]

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INTRODUCTION: There are many threats facing the citrus industry in Florida. Recent freezes in 1983, 1985, and 1989 have pushed the industry southward and closer to Lake Okeechobee. International competition increases yearly with the move toward a global economy. Exotic diseases and pests pose a significant threat as highlighted by the recent 1995 finds of citrus canker Asian strain and the brown citrus aphid. Tristeza, tattler-leaf citrange stunt and the viroids would not be a problem in Florida if the technology to exclude these had been available when citrus production began in Florida.

The vitality, growth and competitiveness of the Florida citrus industry depend on safe access to national and worldwide germplasm resources (Navarro 1986). This requires the collection, conservation and utilization of citrus germplasm. However, the risk inherent with the movement of germplasm into the state must be minimized. The risks of germplasm movement are from citrus graft-transmissible pathogens (CGTP). These include known viruses, viroids, bacteria, *Spiroplasma* and other nonidentified virus-like causal agents (Roistacher 1991). CGTP are often symptomless, and as such, pose a special risk. Risk assessment and testing (indexing) are required to ensure that distributed material is pest-free (Frison 1991).

Limited introduction of new citrus germplasm sources into Florida has occurred during the past 20 years and procedures for the development of virus-free budwood sources have steadily improved over that time. The Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS/DPI) Citrus Germplasm Introduction Program is expanding to triple the capacity for introduction of germplasm that is important for potential commercial utilization and research purposes. This will safely increase the Florida citrus industry's access to worldwide germplasm resources. Crop improvement is needed because 80% of the planted trees are on three of the six commonly used rootstocks. Germplasm is needed for improved cold hardiness, disease and/or nematode tolerance and adaptation to the various Florida climates. The purpose of this circular is to provide a concise explanation of the proper and consistent handling of all citrus introductions into the state. Proper handling minimizes the risks associated with citrus germplasm introductions.

INTRODUCTION OF GERMPLASM: Citrus germplasm, which includes genera of the subfamily Aurantoideae (Rutaceac)(Coile 1995 and Moreno 1993), is prohibited from entering the State of Florida and/or the United States except under a permit. The permitting process for both domestic and foreign germplasm into Florida begins with an application to introduce citrus budwood submitted to the Division of Plant Industry in Gainesville (Form PI-84). Each request is evaluated by the Citrus Budwood Technical Advisory Committee that is composed of six members. Three members are from the citrus industry, and the remaining three represent the administrative offices of (1) FDACS/DPI, Gainesville (2) The Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Lake Alfred, and (3) the U.S. Horticultural Research Laboratory, USDA Agricultural Research Service, Orlando.

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At this stage the division's director can approve entry of domestic germplasm based on committee recommendations. If the committee recommends importing foreign germplasm and if the director approves, a request is submitted to the USDA Animal Plant Health Inspection Service for federal approval. Permitted foreign germplasm must enter through the USDA National Plant Germplasm Center in Maryland for inspection. After inspection for any apparent signs of pests, the germplasm is forwarded to the citrus germplasm introduction facility in Gainesville.

INDEXING STRATEGY: Each cultivar or specimen is treated with a customized program when introduced into the CGIP. (Fig. 1). When the indexing process proves an introduction as free of CGTP, the Division's Bureau of Citrus Budwood Registration maintains and releases the budwood to the industry or to researchers. The indexing strategy is largely based on biological indexing. Quality assurance is the foundation of our program. This includes therapy, individuality, test redundancy, replication, infected controls using mild isolates, healthy controls, and outside review. **Therapy** consists of thermotherapy and microshoot-tip grafting (STG) which initiates the indexing process. This increases the chance of recovering clean germplasm. A minimum of three STG are maintained for each introduction. **Individuality** means that all tests are performed on each STG before release. Test **redundancy** in post-therapy indexing is achieved by using different methods to detect the same pathogen. **Replication** is achieved by requiring citrus indicator plants be grafted with 5-8 viable tissue pieces and at least five herbaceous plants be sap-inoculated for each test. The best environmental conditions for indexing are assured by the symptom expression of mild isolates of CGTP used for **infected controls.** A mild isolate produces symptoms only under the best conditions for pathogenicity. **Outside review** consists of overview by citrus scientists from the University of Florida and the USDA/Agriculture Research Service.

Methods of indexing are classed as citrus indicator tests, herbaceous indicator tests and/or laboratory tests. The strength of a **citrus** indicator, *e.g.* key lime, is its long-term sensitivity to CGTP and the detection of unknown CGTP. A **herbaceous** indicator, such as kidney bean, is a quick test, uses less space, and is good for initial testing and redundancy. The **laboratory tests** are sensitive. Typical laboratory tests include ELISA (enzyme-linked immunosorbent assays), sPAGE (sequential polyacrylamide gel electrophoresis) and nucleic acid hybridization to detect specific CGTP. sPAGE can also detect cryptic (undescribed) viroids. Insect-proof greenhouse facilities with efficient light and temperature controls are required (Frison and Taher 1991).

COLLECTION PROTOCOL: Upon collection and submission of germplasm, an accurate description of the citrus cultivar is required. Information usually collected includes: (1) the general growth conditions and visual health of the plant; (2) a location description of the source plant that enables anyone to locate the plant at a future date; (3) potential pest and pathogen risks existing in the area of origin; and (4) probable or potential CGTP present. The exact location of the source plant becomes important when either a previously undescribed CGTP is detected in the source plant or the area where the plant is located.

When collecting germplasm, tools (clippers, knives, *etc.)* must be sterilized by dipping in 0.5-1.0% a.i. sodium hypochlorite solution. This is followed by thoroughly washing the budwood in soapy water, a 2-3 minute dip in the sodium hypochlorite solution, and a water rinse. Prior to shipping, budwood should be double-wrapped in plastic and properly labeled. Avoid free moisture in the package (Frison and Taher 1991; Roistacher 1991; Wisler *et al.* 1996).

INDEXING PROTOCOL: Indexing after therapy is essential because even the most reliable therapy methods do not work perfectly. There are two indexing protocols available when germplasm is introduced: full and expedited. Choice of protocol is determined by risk assessment. The guidelines for this assessment are outlined below:

FULL PROTOCOL: The full indexing protocol in Fig. 1 indicates the steps necessary to detect all known CGTP and possibly detect undescribed CGTP. The terms necessary to understand the figure are: Cool house - greenhouse (GH) environment maintained between 18-27 C; Hot house - GH environment maintained between 21-35 C; STG - Microshoot-tip grafted plant. The process usually requires 3-5 years for completion. Redundancy is the key, but also the most time-consuming process. More information is found in Wisler *et al.* 1996.

EXPEDITED PROTOCOL: Citrus germplasm available from an institution that recovers and maintains healthy citrus material in an insect-free environment (Frison and Taher 1991) may enter Florida under an expedited testing program. To accomplish this, the practices and procedures of the donor institution must be approved by Florida's Citrus Budwood Technical Advisory Committee. The germplasm then must be certified free of virus and virus-like CGTP's by the donor institution and a record of testing methods employed must accompany the germplasm shipment.
The following testing is required for an expedited entry:

(1) Serological tests for citrus tristeza virus (CTV) and citrus variegation virus (CVV) use tissue from original budwood when it is first received. These tests will be repeated using tissue from inoculated Etrog citron, after the final foliar observation for symptoms has been made on the citron.

(2) Citrus indicator tests for viroids include graft-inoculating Etrog citron and tissue-inoculating Orlando tangelo (OT) with original budstick tissue.

(3) Citrus indicator tests for tatterleaf citrange stunt virus (TL-CSV) use one of the following trifoliates: Swingle citrumelo *(Citrus paradisi x Poncirus trifoliata),* carrizo citrange, Rusk citrange or Troyer citrange *(C. sinensis x P. trifoliata).*

(4) Herbaceous indicator tests use tissue from a tissue-inoculated Orlando tangelo (OT) which has been designated as donor plant for all future tests. The following herbaceous indicators are used: (A) Kidney bean for citrus leaf rugose (CLRV), TL-CSV, and CVV; (B) *Chenopodium quinoa* for citrus ringspot virus (CRSV) and TLCSV.

(5) Molecular viroid testing by sPAGE uses tissue from the graft-inoculated Etrog citron. This testing will be conducted at the Bureau of Citrus Budwood Registration.

CONCLUSIONS: The whole process of indexing exotic germplasm is of critical importance to the introduction of clean germplasm. Many variables with the indexing process such as environment, plant health and cryptic CGTP's demand that our indexing program remain flexible and open to new technologies. Maintaining the flow of economically important varieties and supplies for basic research will keep the industry viable. Growers and the public should be made aware of the importance of this program and the processes of introducing exotic germplasm without introducing exotic pests.

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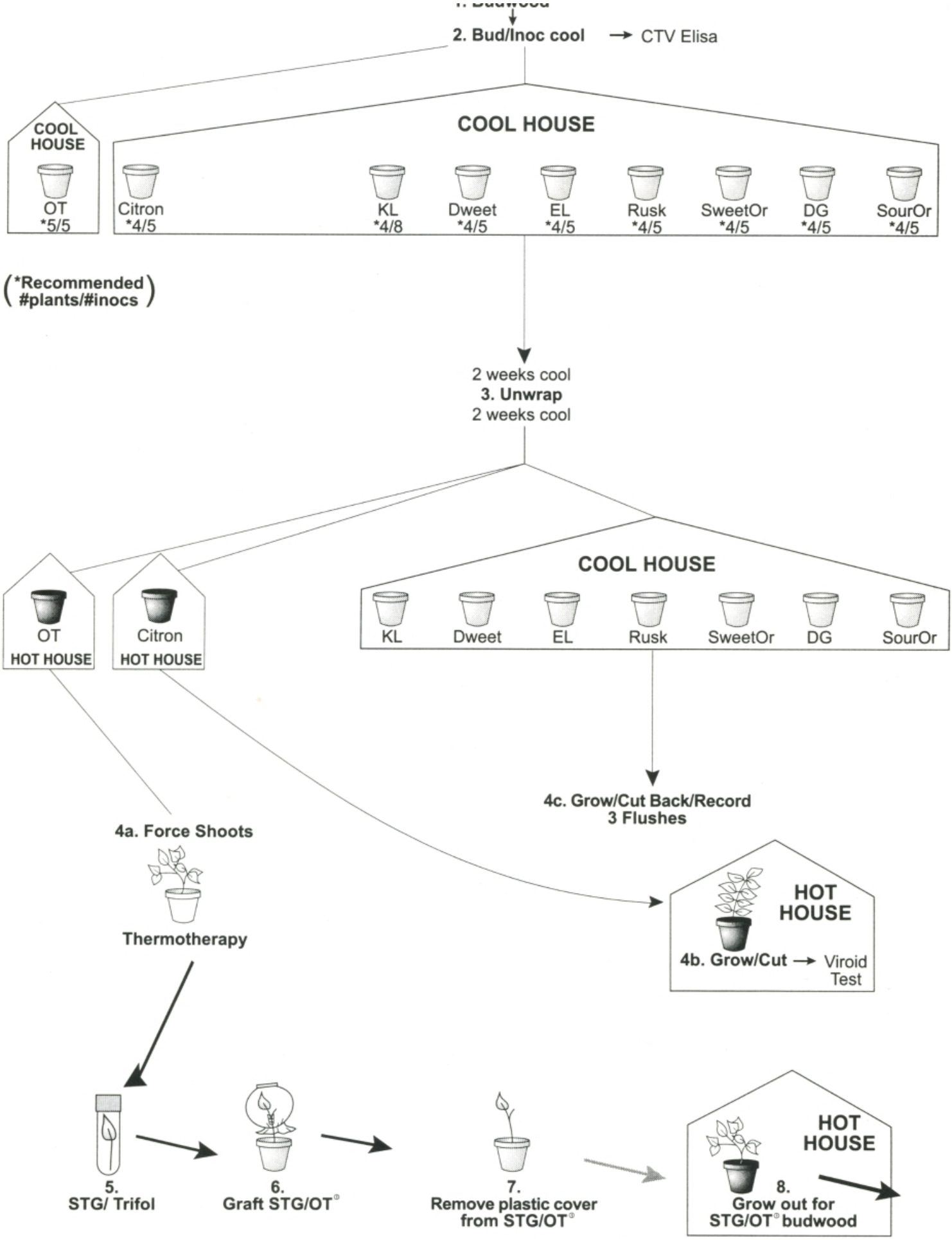
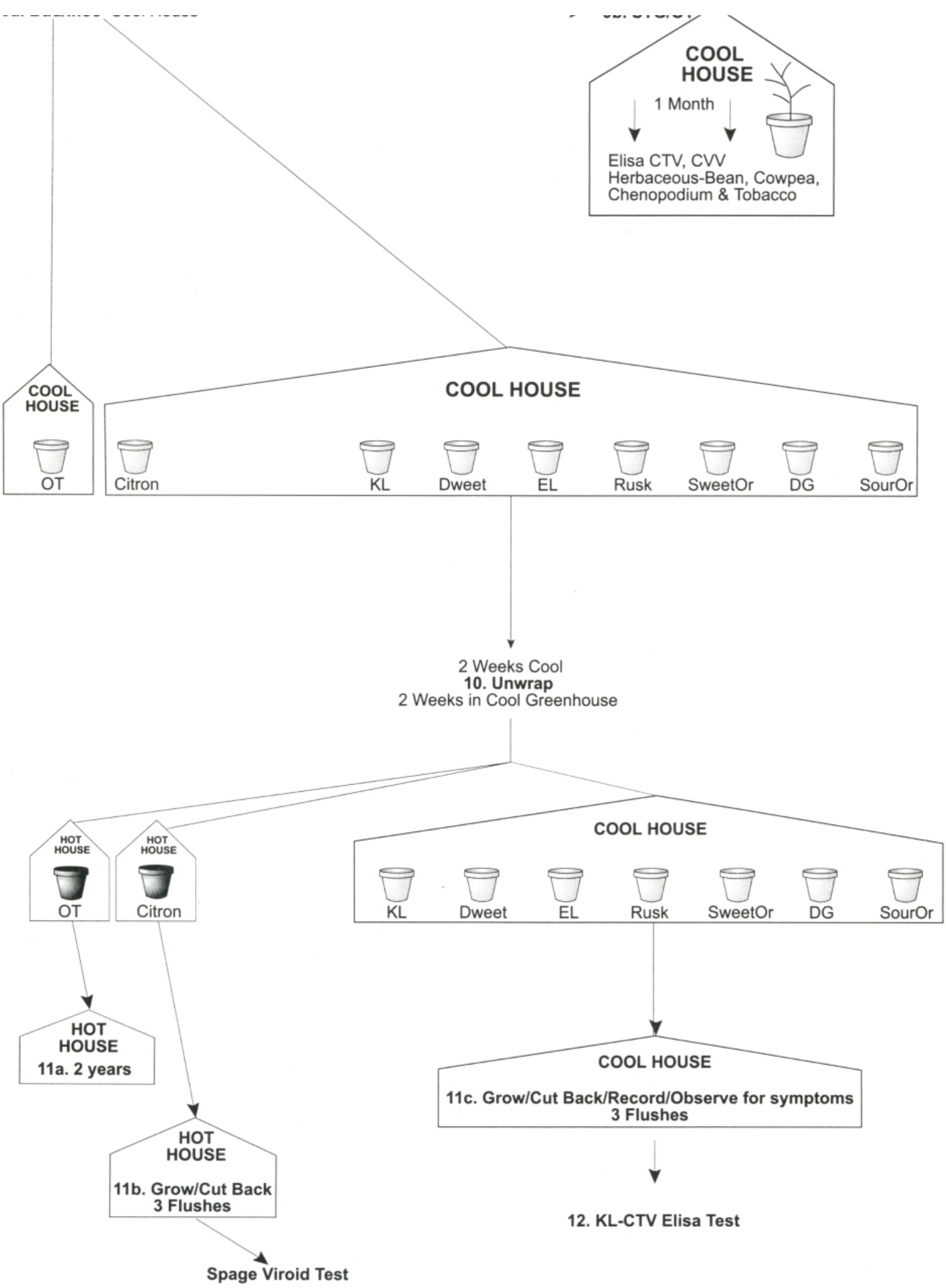


Fig. 1. Full protocol for graft-transmissible pathogens in citrus germplasm. **DG**-Duncan grapefruit, **Dweet**-Dweet tangor, **EL**-Eureka lemon, **KL**-Key lime, **OT**-Orlando tangelo, **Rusk**-Rusk citrange, **SourOr.**-Sour orange; **SweetOr.**-Sweet orange and **Trifol**-trifoliate orange.


COOL HOUSE
1 Month
Elisa CTV, CVV
Herbaceous-Bean, Cowpea, Chenopodium & Tobacco
COOL HOUSE
OT
COOL HOUSE
Citron
KL
Dweet
EL
Rusk
SweetOr
DG
SourOr
2 Weeks Cool
10. Unwrap
2 Weeks in Cool Greenhouse
HOT HOUSE
OT
HOT HOUSE
Citron
COOL HOUSE
KL
Dweet
EL
Rusk
SweetOr
DG
SourOr
HOT HOUSE
11a. 2 years
HOT HOUSE
11b. Grow/Cut Back
3 Flushes
Spage Viroid Test
COOL HOUSE
11c. Grow/Cut Back/Record/Observe for symptoms
3 Flushes
12. KL-CTV Elisa Test